

# SEQUENCE LISTING

<110> MILLER, JEFFREY C.  
 ZHANG, LEI

<120> METHODS AND COMPOSITIONS FOR TARGETED CLEAVAGE AND RECOMBINATION

<130> 8325-0036.31

<140> 10/587,723  
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 <151> 2004-03-26

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 <151> 2004-06-01

<150> 10/912,932  
 <151> 2004-08-06

<160> 212

<170> PatentIn version 3.3

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 <222> (5)..(16)  
 <223> Target sequence for the hSMC1-specific ZFP

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 <222> (23)..(34)  
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<213> Artificial

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ggtcgcgagg agcaagtgtt tggtgttgcc atgcaacaag aaaagggggc ggaggcacca 180  
cgccagtcgt cagctcgctc ctcgatatag caacatcagt ccccgcccct ggtcccactc 240

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cctgtcctac tgctgccggc gccgcggccg tcataagaag cttcctgaaa ctgattgaag      420
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<223> Target sequence for the second pair of IL2Rgamma-specific ZFP

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<223> Target sequence for the second pair of IL2Rgamma-specific ZFP

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<400> 8

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<220>
<223> IL2Rgamma gene

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<212> DNA  
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<220>  
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<400> 11  
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<223> mutated IL2Rgamma gene

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gcaaaaaaag gagatccacc tctaccaaac atttgttggt cagctccagg acccacggga 180  
accaggaga caggccacac agatgctaaa actgcagaat ctgggtaatt tggaaagaaa 240  
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taggggcact accttcagga tcctgacttg tctaggccag gggaatgacc acatatgcac 420  
acatatctcc agtgatcccc tgggctccag agaacctaac acttcacaaa ctgagtgaat 480  
cccagctaga actgaactgg aacaacagat tcttgaacca ctgtttggag cacttggtgc 540  
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attacaattc actggccgctc gttt 624

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<212> DNA  
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<220>  
 <223> human beta-globin gene

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 actcctaagc cagtgccaga agagccaagg acaggtacgg ctgtcatcac ttagacctca 120  
 ccctgtggag ccacacccta gggttggcca atctactccc aggagcaggg agggcaggag 180  
 ccagggctgg gcataaaagt cagggcagag ccatctattg cttacatttg cttctgacac 240  
 aactgtgttc actagcaacc tcaaacagac accatggtgc atctgactcc tgaggagaag 300  
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 ttttcccacc cttaggctgc tgggtgtcta cccttgacc cagaggttct ttgagtcctt 540  
 tggggatctg tccactcctg atgctgttat gggcaaccct aaggtgaagg ctcatggcaa 600  
 gaaagtgctc ggtgccttta gtgatggcct ggctcacctg gacaacctca agggcacctt 660  
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 <212> DNA  
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<220>  
 <223> an amplification product derived from a mutated beta-globin gene

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 agtagcaatt tgtactgatg gtatggggcc aagagatata tcttagaggg agggctgagg 120  
 gtttgaagtc caactcctaa gccagtcca gaagagccaa ggacaggtac ggctgtcatc 180  
 acttagacct caccctgtgg agccacaccc taggggtggc caatctactc ccaggagcag 240  
 ggagggcagg agccagggct gggcataaaa gtcagggcag agccatctat tgcttacatt 300  
 tgcttctgac acaactgtgt tcactagcaa cctcaaacag acaccatggg gcatctgact 360  
 cctgaggaga agtctggcgt tagtgcccga attccgatcg tcaaccac 408

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<220>  
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 <223> target sequences for the 5-8 ZFP/FokI fusion protein

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42

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<220>  
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 <222> (1)..(17)  
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<220>  
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<220>  
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 <223> ZC linker

<220>  
 <221> MISC\_FEATURE  
 <222> (141)..(336)  
 <223> FokI cleavage half-domain

<400> 16

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 20 25 30

Ser Arg Ser Asp Asn Leu Ser Glu His Ile Arg Thr His Thr Gly Glu  
 35 40 45

Lys Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Arg Asn Ala  
 50 55 60

His Arg Ile Asn His Thr Lys Ile His Thr Gly Ser Gln Lys Pro Phe  
 65 70 75 80

Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp Thr Leu Ser  
 85 90 95

Glu His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile  
 100 105 110

Cys Gly Arg Lys Phe Ala Ala Arg Ser Thr Arg Thr Thr His Thr Lys  
 115 120 125

Ile His Leu Arg Gln Lys Asp Ala Ala Arg Gly Ser Gln Leu Val Lys  
 130 135 140

Ser Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg His Lys Leu Lys Tyr  
 145 150 155 160

Val Pro His Glu Tyr Ile Glu Leu Ile Glu Ile Ala Arg Asn Ser Thr  
 165 170 175

Gln Asp Arg Ile Leu Glu Met Lys Val Met Glu Phe Phe Met Lys Val  
 180 185 190

Tyr Gly Tyr Arg Gly Lys His Leu Gly Gly Ser Arg Lys Pro Asp Gly  
 195 200 205

Ala Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr Gly Val Ile Val Asp  
 210 215 220

Thr Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro Ile Gly Gln Ala Asp  
 225 230 235 240

Glu Met Gln Arg Tyr Val Glu Glu Asn Gln Thr Arg Asn Lys His Ile  
 245 250 255

Asn Pro Asn Glu Trp Trp Lys Val Tyr Pro Ser Ser Val Thr Glu Phe  
 260 265 270

Lys Phe Leu Phe Val Ser Gly His Phe Lys Gly Asn Tyr Lys Ala Gln  
 275 280 285

Leu Thr Arg Leu Asn His Ile Thr Asn Cys Asn Gly Ala Val Leu Ser  
 290 295 300

Val Glu Glu Leu Leu Ile Gly Gly Glu Met Ile Lys Ala Gly Thr Leu  
 305 310 315 320

Thr Leu Glu Glu Val Arg Arg Lys Phe Asn Asn Gly Glu Ile Asn Phe  
 325 330 335

<210> 17  
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 <212> PRT  
 <213> Artificial

<220>  
 <223> 5-10 ZFP/FokI fusion

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<220>  
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 1 5 10 15

Ala Met Ala Glu Arg Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe  
 20 25 30

Ser Arg Ser Asp Ser Leu Ser Arg His Ile Arg Thr His Thr Gly Glu  
 35 40 45

Lys Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Asp Ser Ser  
 50 55 60

Asn Arg Lys Thr His Thr Lys Ile His Thr Gly Gly Gly Gly Ser Gln  
 65 70 75 80

Lys Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp  
 85 90 95



Ser Leu Ser Val His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala  
100 105 110

Cys Asp Ile Cys Gly Arg Lys Phe Ala Asp Arg Ser Asn Arg Ile Thr  
115 120 125

His Thr Lys Ile His Leu Arg Gln Lys Asp Ala Ala Arg Gly Ser Gln  
130 135 140

Leu Val Lys Ser Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg His Lys  
145 150 155 160

Leu Lys Tyr Val Pro His Glu Tyr Ile Glu Leu Ile Glu Ile Ala Arg  
165 170 175

Asn Ser Thr Gln Asp Arg Ile Leu Glu Met Lys Val Met Glu Phe Phe  
180 185 190

Met Lys Val Tyr Gly Tyr Arg Gly Lys His Leu Gly Gly Ser Arg Lys  
195 200 205

Pro Asp Gly Ala Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr Gly Val  
210 215 220

Ile Val Asp Thr Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro Ile Gly  
225 230 235 240

Gln Ala Asp Glu Met Gln Arg Tyr Val Glu Glu Asn Gln Thr Arg Asn  
245 250 255

Lys His Ile Asn Pro Asn Glu Trp Trp Lys Val Tyr Pro Ser Ser Val  
260 265 270

Thr Glu Phe Lys Phe Leu Phe Val Ser Gly His Phe Lys Gly Asn Tyr  
275 280 285

Lys Ala Gln Leu Thr Arg Leu Asn His Ile Thr Asn Cys Asn Gly Ala  
290 295 300

Val Leu Ser Val Glu Glu Leu Leu Ile Gly Gly Glu Met Ile Lys Ala  
305 310 315 320

Gly Thr Leu Thr Leu Glu Glu Val Arg Arg Lys Phe Asn Asn Gly Glu  
325 330 335

Ile Asn Phe

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 <211> 797  
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 <213> Artificial

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 aacggccaca agttcagcgt gtccggcgag ggcgagggcg atgccaccta cggcaagctg 180  
 accctgaagt tcatctgcac caccggcaag ctgcccgtgc cctggcccac cctcgtgacc 240  
 accctgacct acggcggtgca gtgcttcagc cgctaccccg accacatgaa gcagcacgac 300  
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 gacggcaact acaagacccg cgccgaggtg aagtctgagg gcgacaccct ggtgaaccgc 420  
 atcgagctga agggcatcga cttcaaggag gacggcaaca tcctggggca caagctggag 480  
 tacaactaca acagccacaa cgtctatata atggccgaca agcagaagaa cggcatcaag 540  
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 cagcagaaca cccccatcgg cgacggcccc gtgctgctgc ccgacaacca ctacctgagc 660  
 acccagtcgg ccctgagcaa agaccccaac gagaagcgcg atcacatggt cctgctggag 720  
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 gactctagat cataatc 797

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 <211> 795  
 <212> DNA  
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 <223> mutant defective eGFP gene

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 aacggccaca agttcagcgt gtccggcgag ggcgagggcg atgccaccta cggcaagctg 180  
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cttcaagtcc gccatgcccg aaggctacgt ccaggagcgc accatcttct tcaaggacga	360
cggcaactac aagacccgcg ccgaggtgaa gtctgagggc gacaccctgg tgaaccgcat	420
cgagctgaag ggcacgcact tcaaggagga cggcaacatc ctggggcaca agctggagta	480
caactacaac agccacaacg tctatatcat ggccgacaag cagaagaacg gcatcaaggt	540
gaacttcaag atccgccaca acatcgagga cggcagcgtg cagctcgccg accactacca	600
gcagaacacc cccatcggcg acggccccgt gctgctgccc gacaaccact acctgagcac	660
ccagtcgcgc ctgagcaaag accccaacga gaagcgcgat cacatggtcc tgctggagtt	720
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ctctagatca taatc	795

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ctgaagttca tctgcaccac cggcaagctg cccgtgccct ggccccaccct cgtgaccacc	180
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ttcaagtccg ccatgcccga aggctacgtc caggagcgc ccatcttctt caaggacgac	300
ggcaactaca agacccgcgc cgaggtgaag ttcgagggcg acaccctggt gaaccgcac	360
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cagaacaccc ccatcggcga cggccccgtg ctgctgcccg acaaccacta cctgagcacc	600
cagtccgccc tgagcaaaga cccaacgag aagcgcgatc acatggtcct gctggagttc	660
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tctagatcat aatc	734

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<223> eGFP insert in pCR(R)4-TOPO

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ggcaactaca	agaccgcgc	cgaggtgaag	ttcgagggcg	acaccctggt	gaaccgcac	360
gagctgaagg	gcacgcactt	caaggaggac	ggcaacatcc	tggggcacia	gctggagtac	420
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aacttcaaga	tccgccacia	catcgaggac	ggcagcgtgc	agctcgccga	ccactaccag	540
cagaacaccc	ccatcggcga	cggccccgtg	ctgctgcccg	acaaccacta	cctgagcacc	600
cagtccgccc	tgagcaaaga	ccccaacgag	aagcgcgac	acatggtcct	gctggagttc	660
gtgaccgccc	ccgggatcac	tctcggcatg	gacgagctgt	acaagtaaag	cggccgctcg	720
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ttaatagtgg	actcttggtc	caaactggaa	caacactcaa	ccctatctcg	gtctattctt	1380
ttgatttata	agggattttg	ccgatttcgg	cctattgggt	aaaaaatgag	ctgatttaac	1440
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<210> 22

<211> 116

<212> PRT

<213> Artificial

<220>

<223> sca-29b

<400> 22

Met Ala Glu Arg Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser  
1 5 10 15

Gln Ser Gly Asp Leu Thr Arg His Ile Arg Thr His Thr Gly Glu Lys  
20 25 30

Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Thr Ser Ala Asn  
35 40 45

Leu Ser Arg His Thr Lys Ile His Thr Gly Gly Gly Ser Gln Lys  
50 55 60

Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Arg Ser Ala  
65 70 75 80

Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys  
85 90 95

Asp Ile Cys Gly Arg Lys Phe Ala Gln Ser Gly His Leu Ser Arg His  
100 105 110

Thr Lys Ile His  
115

<210> 23

<211> 113

<212> PRT

<213> Artificial

<220>

<223> sca-36a

<400> 23

Met Ala Glu Arg Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser  
1 5 10 15

Arg Ser Gln Thr Arg Lys Thr His Ile Arg Thr His Thr Gly Glu Lys  
20 25 30

Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Gln Lys Arg Asn  
35 40 45

Arg Thr Lys His Thr Lys Ile His Thr Gly Ser Gln Lys Pro Phe Gln

50	55	60
Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Arg Ser Ala Leu Ser Arg		
65	70	75 80
His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile Cys		
	85 90	95
Gly Arg Lys Phe Ala Gln Ser Gly Asn Leu Ala Arg His Thr Lys Ile		
	100 105	110

His

<210> 24  
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<220>  
 <223> sca-36b

<400> 24

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Thr Ser Gly Ser Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys	
20 25 30	
Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Asp Arg Ser Asp	
35 40 45	
Leu Ser Arg His Thr Lys Ile His Thr Gly Gly Gly Gly Ser Gln Lys	
50 55 60	
Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Arg Ser Ala	
65 70 75 80	
Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys	
85 90 95	
Asp Ile Cys Gly Arg Lys Phe Ala Gln Ser Gly Asn Leu Ala Arg His	
100 105 110	
Thr Lys Ile His	
115	

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<220>  
 <223> sca-36c

<400> 25

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 1 5 10 15

Thr Ser Ser Ser Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys  
 20 25 30

Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Asp Arg Ser Asp  
 35 40 45

Leu Ser Arg His Thr Lys Ile His Thr Gly Gly Gly Gly Ser Gln Lys  
 50 55 60

Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Arg Ser Ala  
 65 70 75 80

Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys  
 85 90 95

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Arg Ile Asn His Thr Lys Ile His Thr Gly Ser Gln Lys Pro Phe Gln  
 50 55 60

Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp Thr Leu Ser Glu  
 65 70 75 80

His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile Cys  
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Gly Arg Lys Phe Ala Ala Arg Ser Thr Arg Thr Asn His Thr Lys Ile  
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Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Ala Arg Ser Thr  
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Arg Thr Thr His Thr Lys Ile His Thr Gly Ser Gln Lys Pro Phe Gln  
 50 55 60

Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp Ser Leu Ser Lys  
 65 70 75 80

His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile Cys  
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